CCNA2 Deletion Decreases Induced Neurons through 3D Nanochannel Electroporation

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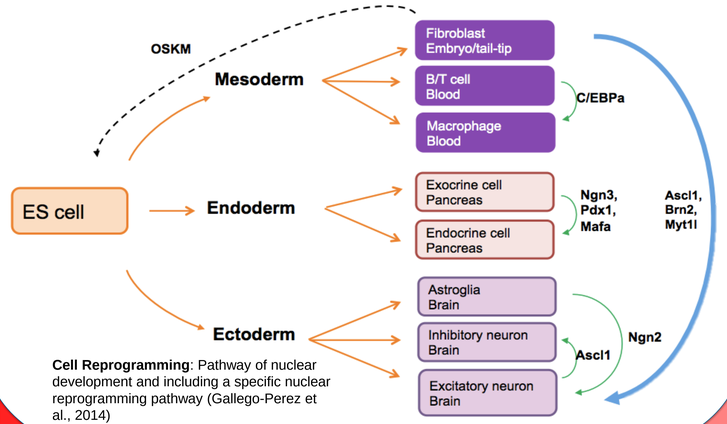
Background: Cell Reprogramming

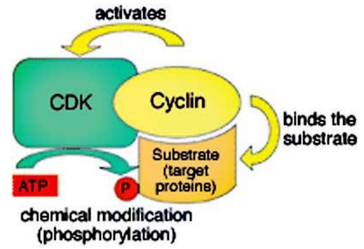
- Cell reprogramming is a switch in gene expression of a specialized cell into an entirely different cell type



Cell Reprogramming: Pathway of nuclear development and including a specific nuclear reprogramming pathway (Gallego-Perez et al., 2014)

Background and Methods: *CCNA2*

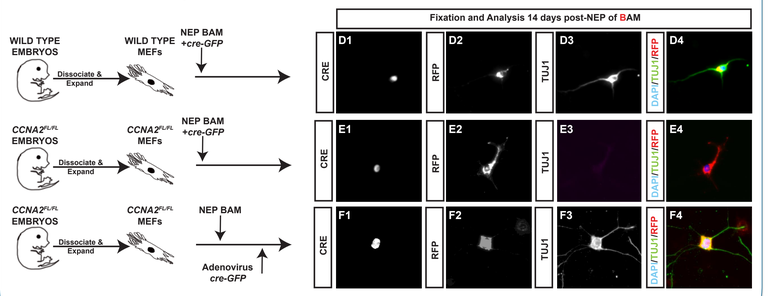
- Cyclins are proteins that regulate the cell cycle by activating cyclin-dependent kinases (CDKs)
- CDKs then phosphorylate proteins necessary for the cell cycle
- Cyclin A2, from the gene *CCNA2*, has been shown in previous research studies to be necessary to begin DNA replication, particularly the S-phase
- Deletion of the *CCNA2* gene locus was done by cre-mediated recombination of a *CCNA2*-floxed allele.
- It's effect on nuclear repro-gramming can be measured by the amount of TUJ1-positive cells



CDK-Cyclin Complex: When cyclin binds to CDK, the kinase is activated and can phosphorylate the target protein (Alberts et al., 2002)

Results of *CCNA2*

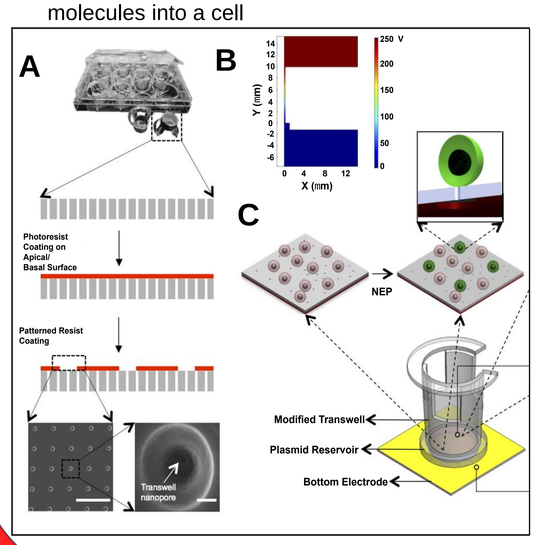
- *CCNA2* is required for BAM-mediated induced neuronal formation



Early deletion of *CCNA2* blocks neuronal reprogramming: Experimental schematics are illustrated to the left of each panel. Molecular markers are denoted on the left of each panel. Images are epifluorescent photomicrographs.

Background and Methods: NEP

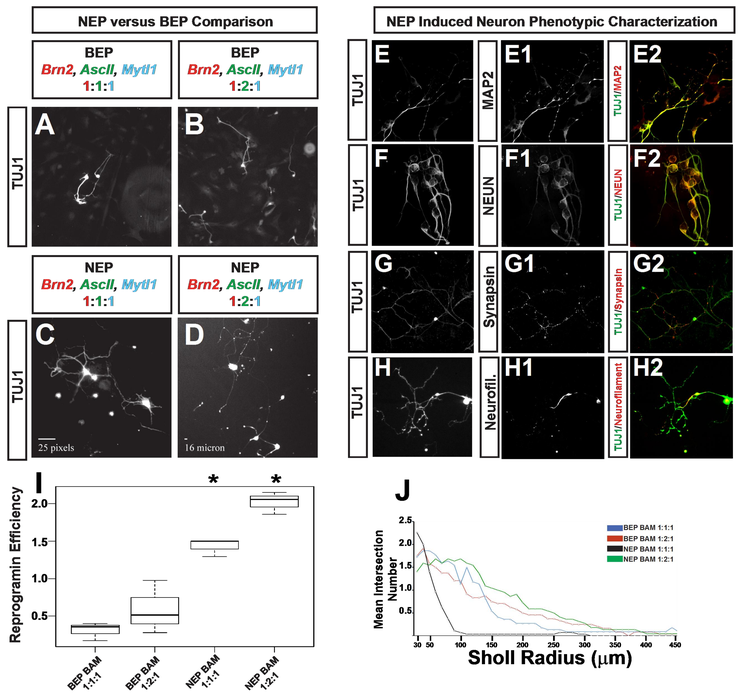
- Cell reprogramming can be done by transfection (introducing DNA into cells) traditionally done by bulk electroporation (BEP)
- Nanochannel electroporation (NEP) is a novel method to transfect
- NEP is optimal for gene delivery due to the capability of introducing complex combinations of DNA into a large number of individual cells
- Conditions can be adjusted to control the amount of molecules delivered: high transfection efficiencies and low cell-to-cell variability
- Achieved by applying a focused electric field through a nanochannel, which electrophoretically nanoporates and delivers precisely charged molecules into a cell



3D NEP:
(A) Transwell modification steps with scanning electron micrographs of the microwell array.
(B) Circuit illustration of the resistance distribution
(C) Schematic diagram illus-trating the NEP process, with image of a transfected/green cell in direct contact with an open Transwell nanochannel. Gallego-Perez et al., to be published)

Results of NEP

- NEP can uniquely control the plasmid ratio during reprograming



Induced Neuron Formation by NEP: (A-D) Epifluorscent photomicrographs of TUJ1 stained cells 7 days post BEP or NEP using differing ratios of BAM. (E-H) Phenotypic characterization of induced neurons post-NEP at 14 days. (I) Neuronal reprograming efficiency is plotted as a function of electroporation condition. (J) Neuronal complexity index of induced neurons by Sholl Radius analysis. (Gallego-Perez et al., to be published)

Significance

- Regenerative medicine is a clinical application of research that replaces human cells or organs to restore normal function, often with stem cells
- Researchers can regenerate damaged tissues and organs or stimulate the body's own repair mechanisms
- Directed reprogramming into specific cell lineages has results directly applicable to clinical studies with regenerative medicine
- Future work: since *Ascl1* had the greatest effect on neuronal reprogramming of the three transcription factors, perhaps *Ascl1* requires *CCNA2* to function

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Wexner Medical Center